Figure 1: Biosynthetic scheme of carotenoids in tomato flowers

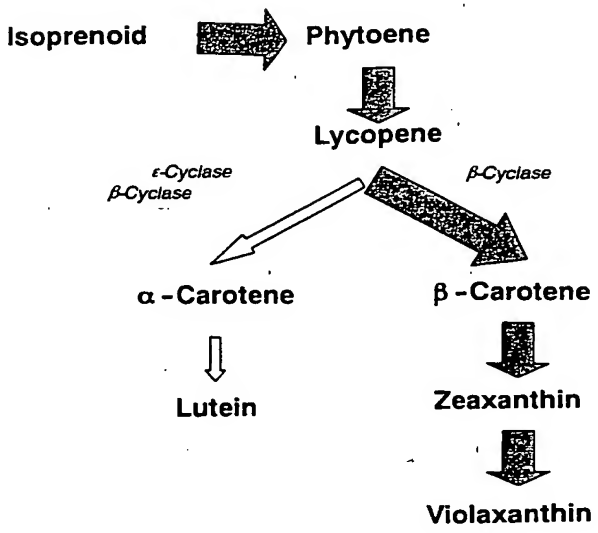


Figure 2: Biosynthetic scheme of Astaxanthin in genetically modified flowers

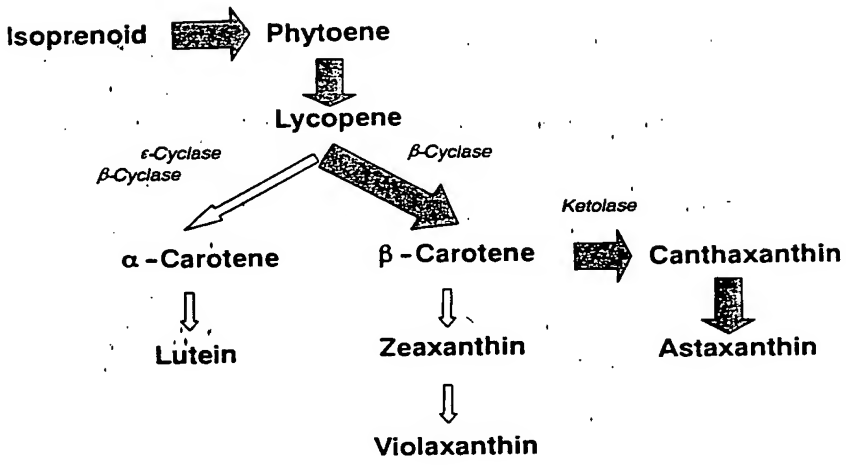


Figure 3: Nucleotide sequence alignment

Figure 4: Protein sequence alignment

```
KETO2.pro   MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATQYSLPSEESDAA 50
X86782.pro  MQLAATVMLEQLTGSAEALKEKEKEVAGSSDVLRTWATQYSLPSEESDAA 50

KETO2.pro   RPGLKNAYKPPPSDTKGITMALAVIGSWAAVFLHAIFQIKLPTSLDQLHW 100
X86782.pro  RPGLKNAYKPPPSDTKGITMALRVIGSWAAVFLHAIFQIKLPTSLDQLHW 100

KETO2.pro   LPVSDATAQLVSGSSSLLHIVVVFFVLEFLYTGLFITTHDAMHGTIAMRN 150
X86782.pro  LPVSDATAQLVSGTSSLLDIVVVFFVLEFLYTGLFITTHDAMHGTIAMRN 150

KETO2.pro   RQLNDFLGRVCISLYAWFDYNMLHRKHWEHHNHTGEVGKDPDFHRGNPGI 200
X86782.pro  RQLNDFLGRVCISLYAWFDYNMLHRKHWEHHNHTGEVGKDPDFHRGNPGI 200

KETO2.pro   VPWFASFMSSYMSMWQFARLAWWTVVMQLLGAPMANLLVFMAAAPILSAF 250
X86782.pro  VPWFASFMSSYMSMWQFARLAWWTVVMQLLGAPMANLLVFMAAAPILSAF 250

KETO2.pro   RLFYFGTYMPHKPEPGAASGSSPAVMNWWKSRTSQASDLVSFLTCYHFDL 300
X86782.pro  RLFYFGTYMPHKPEPGAASGSSPAVMNWWKSRTSQASDLVSFLTCYHFDL 300

KETO2.pro   HWEHHRWPFAPWWELPNCRRLSGRGLVPA                     329
X86782.pro  HWEHHRWPFAPWWELPNCRRLSGRGLVPA                     329
```

Figure 5A: Construct for overexpressing the ketolase (β-C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea under the control of the d35S-promoter (tomato transformation construct)

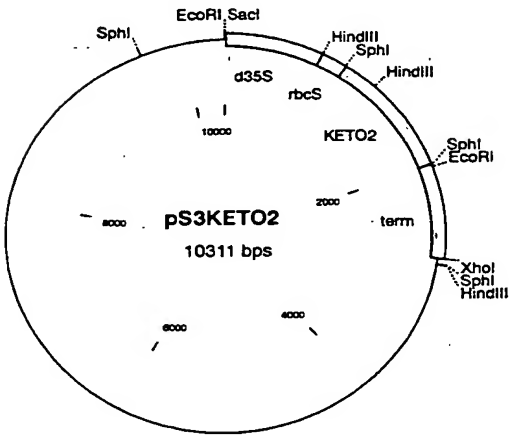


Figure 5B: Construct for overexpressing the ketolase (β-C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea under the control of the d35S-promoter (Tagetes transformation construct)

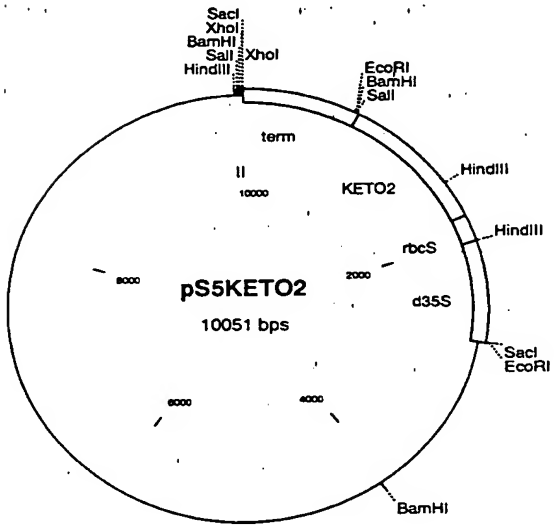


Figure 6: Construct for overexpressing the N-terminally truncated ketolase (β-C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea under the control of the d35S promoter.

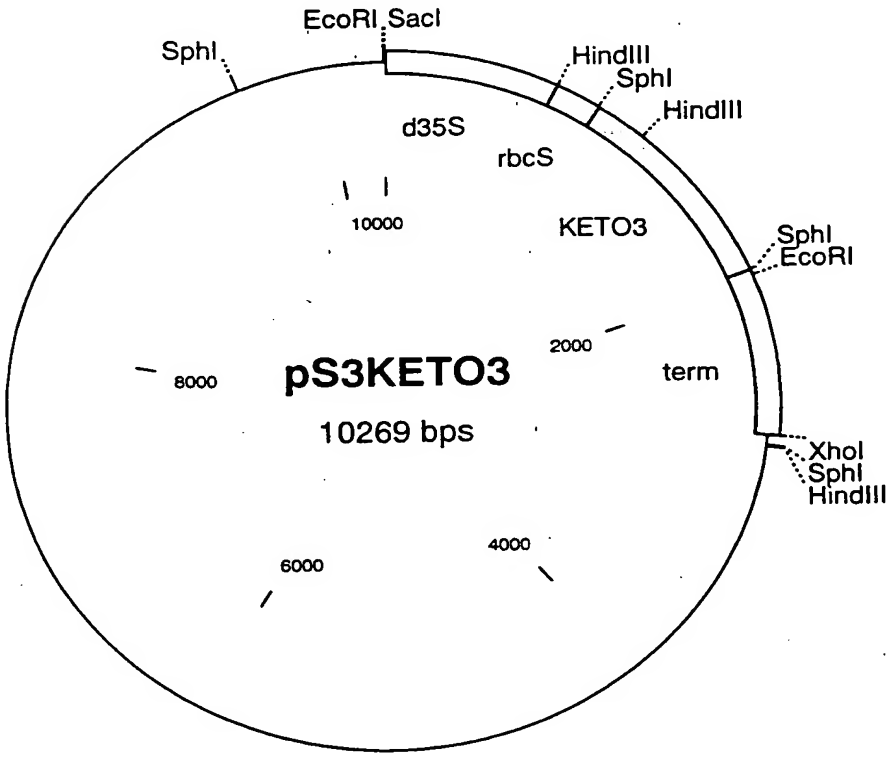


Figure 7: Construct for overexpressing the ketolase (β-C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea and C-terminal myc tag under the control of the d35S promoter.

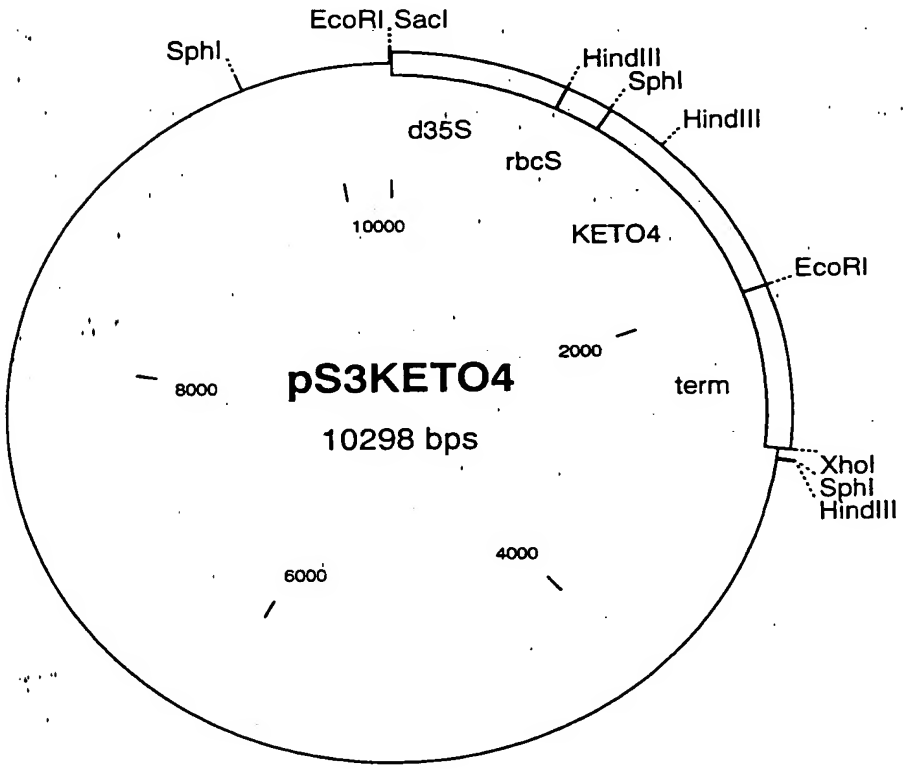


Figure 8A: Construct pS3AP3PKETO2 for overexpressing the ketolase (β-C-4-oxygenase) proteins from H. pluvialis with rbcS transit peptide from pea under the control of the AP3P promoter (tomato transformation construct).

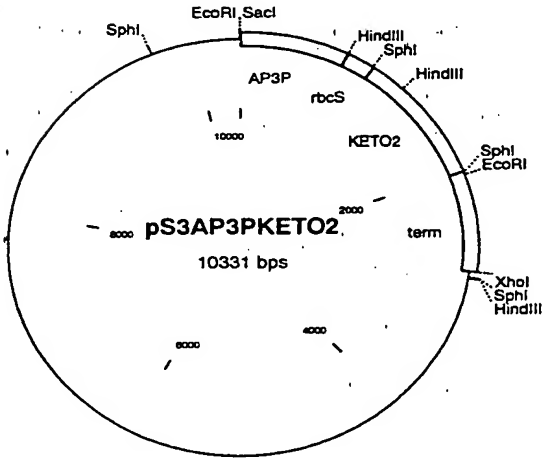


Figure 8B: Construct pS3AP3PKETO2 for overexpressing the ketolase (β-C-4-oxygenase) proteins from H. pluvialis with rbcS transit peptide from pea under the control of the AP3P promoter (Tagetes transformation construct).

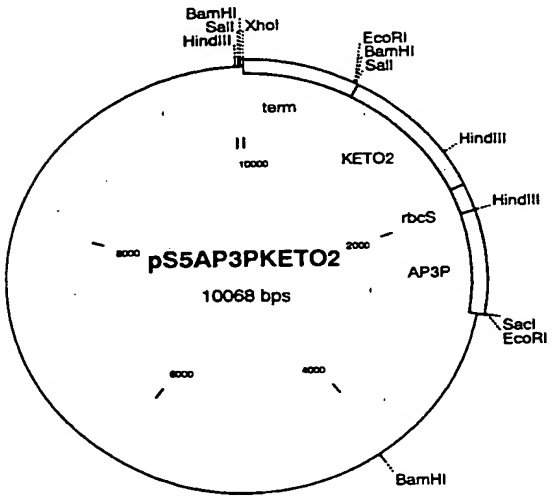


Figure 9: Construct for overexpressing the ketolase (β-C-4-oxygenase) protein from *H. pluvialis* with rbcS transit peptide from pea and C-terminal myc tag under the control of the AP3P promoter.

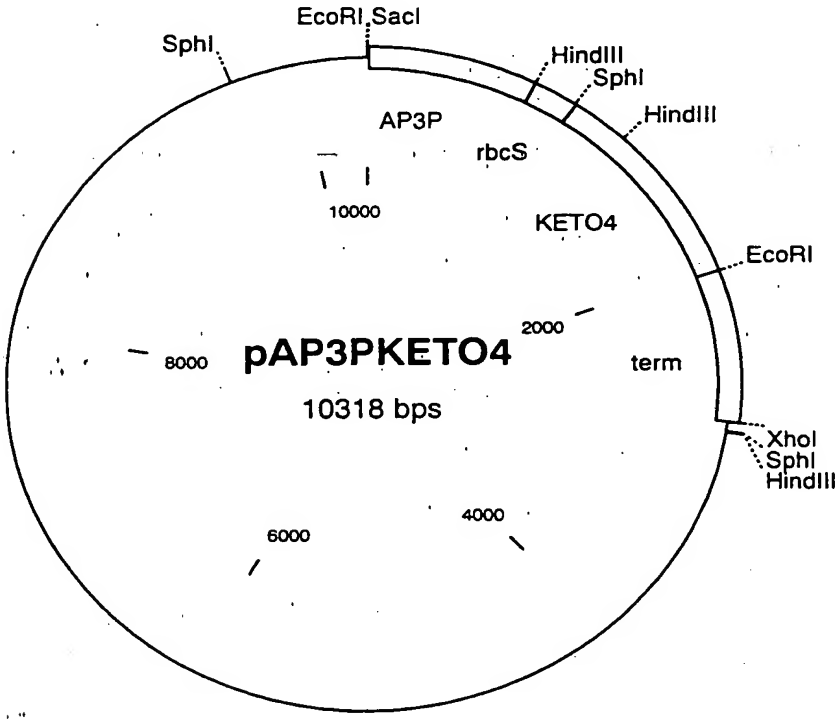
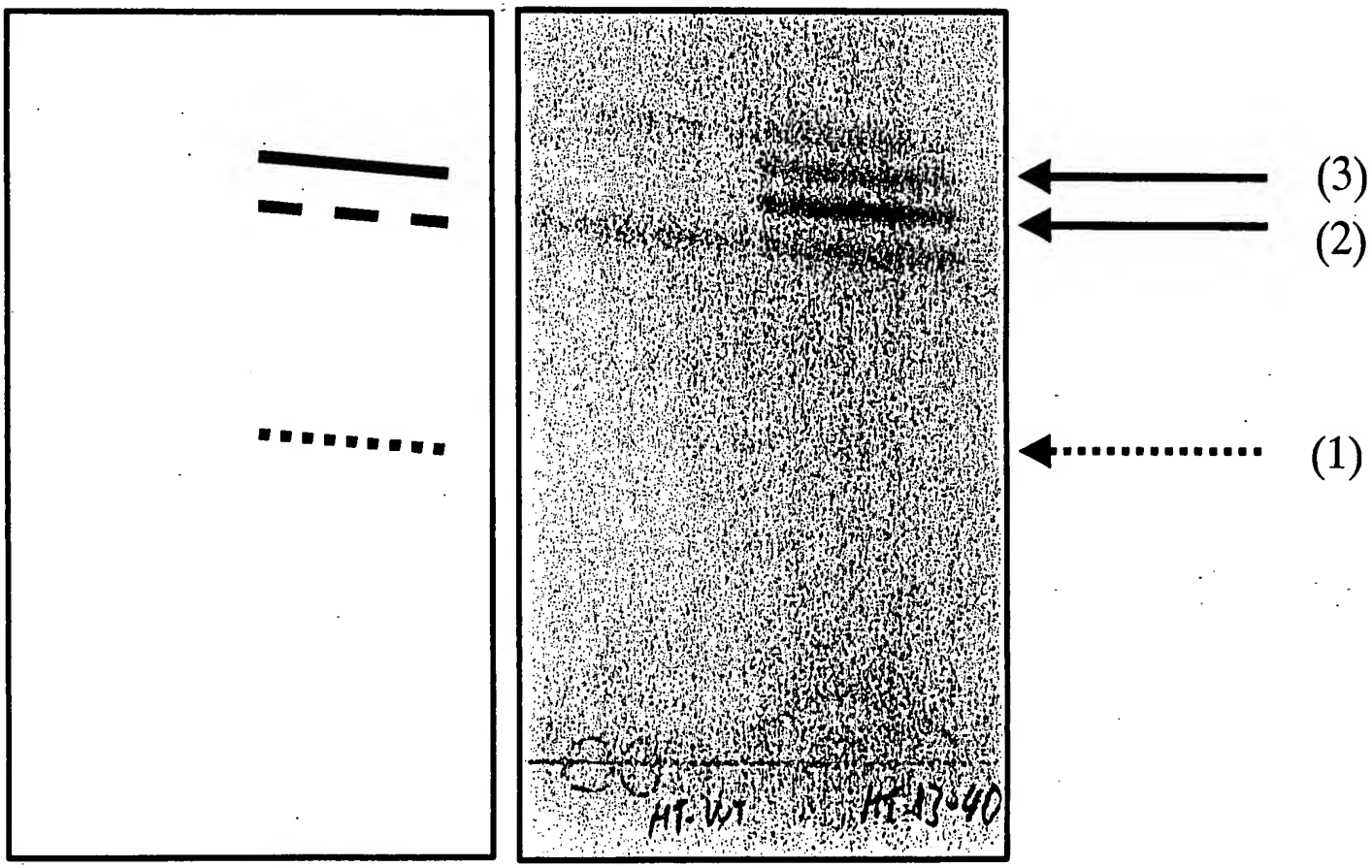


Figure 9a: Ester separation by means of thin-layer chromatography



Left: schematic representation; right: photograph of the thin-layer plate. (3) and (2) indicate ketocarotenoid diesters, (1) ketocarotenoid monoesters

Fig. 10

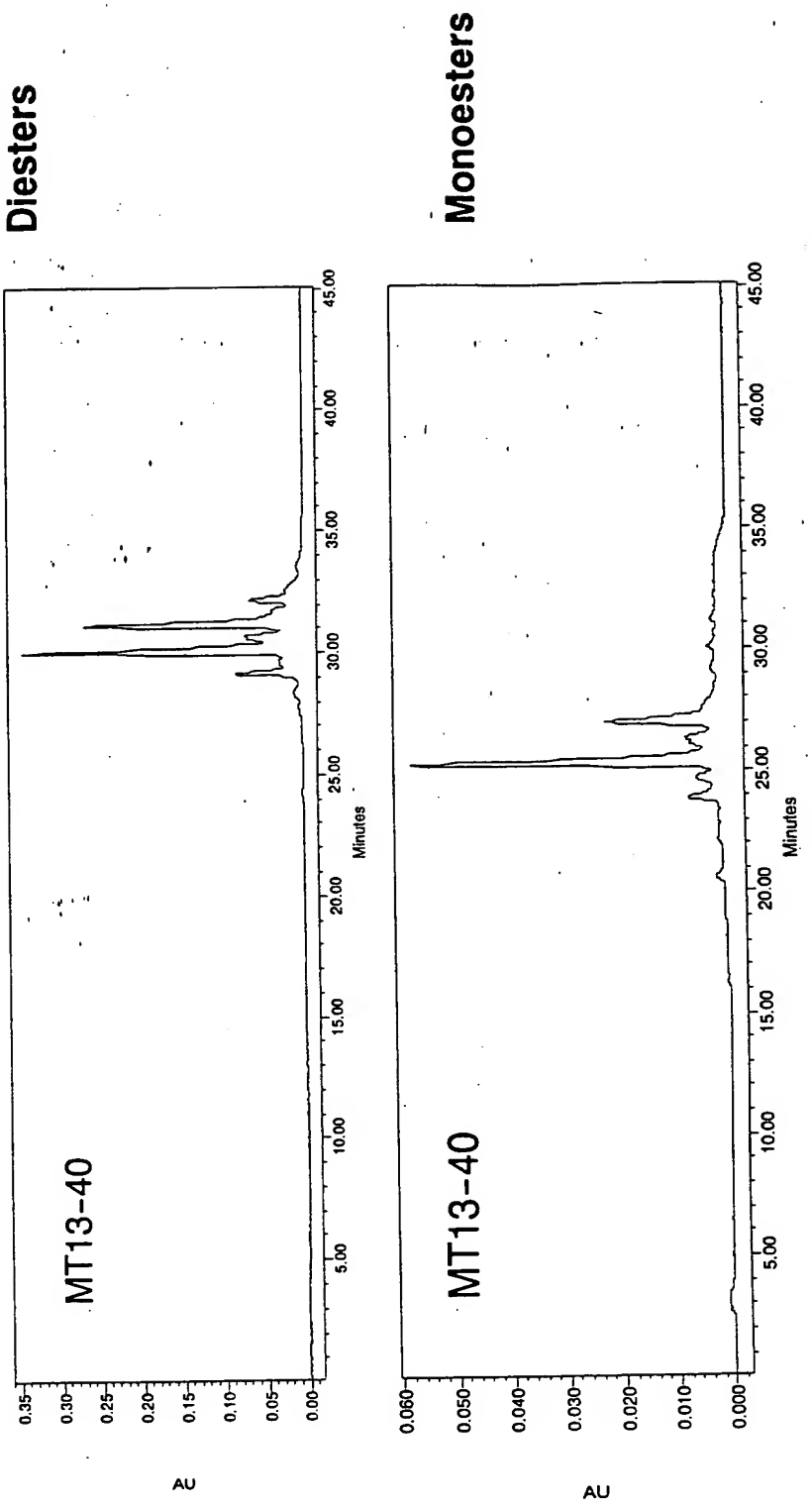


Fig. 11

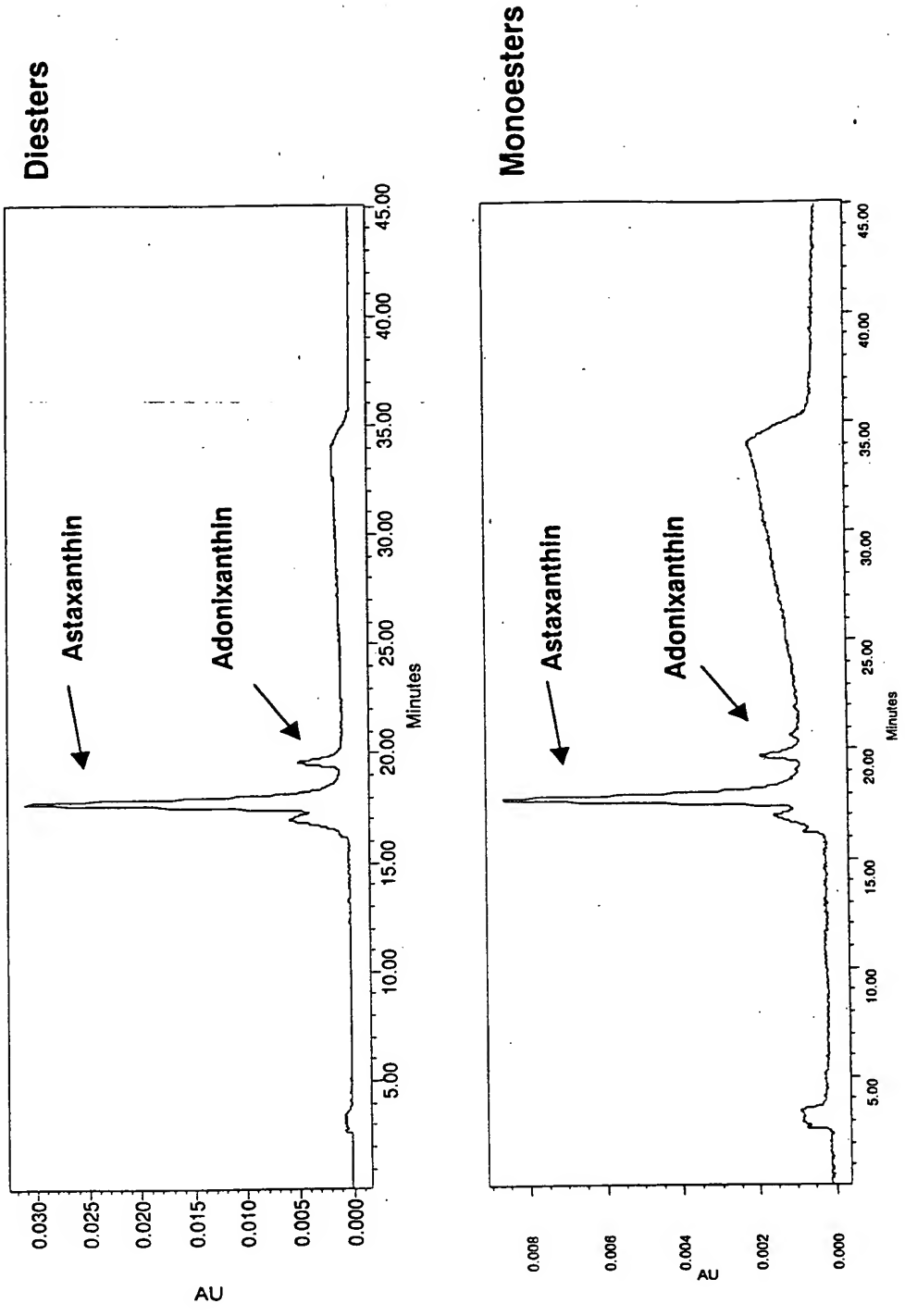
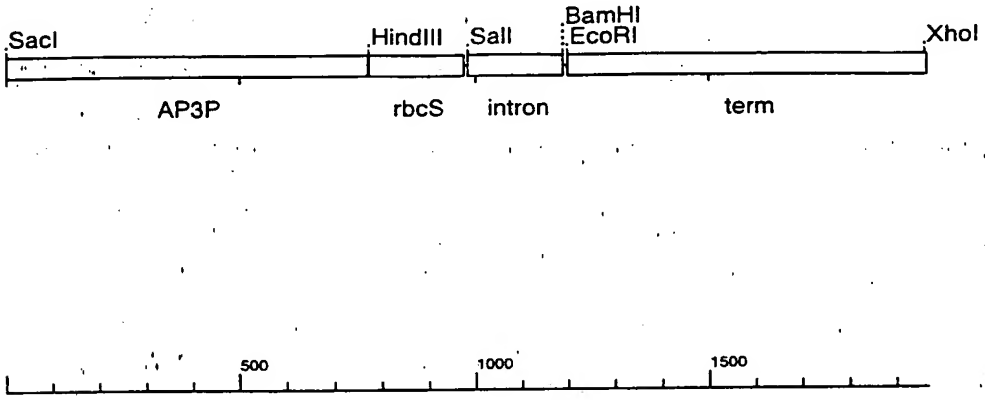

Diesters
Astaxanthin
Adonixanthin
AU
Minutes
0.030
0.025
0.020
0.015
0.010
0.005
0.000
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
Monoesters
Astaxanthin
Adonixanthin
AU
Minutes
0.008
0.006
0.004
0.002
0.000

Figure 12: Cloning cassette for the preparation of inverted-repeat expression cassettes for the flower-specific expression of epsilon-cyclase dsRNAs in Tagetes erecta



pJAI1 (1966 bps)

Figure 13: Expression vector for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter

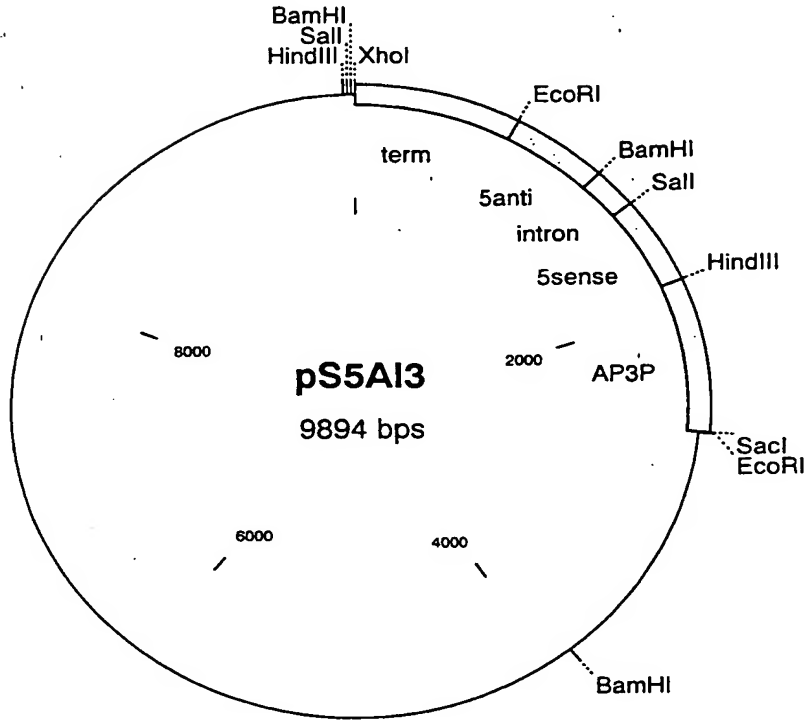


Figure 14: Expression vector for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the CHRC promoter

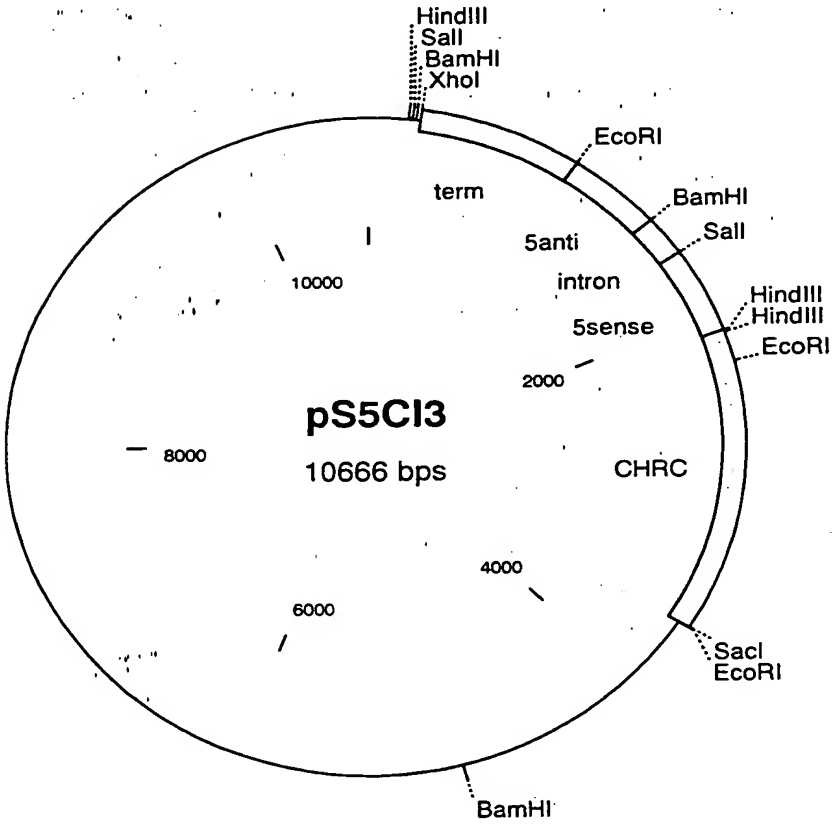


Figure 15: Expression vector for the flower-specific production of dsRNA transcripts comprising 3'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter

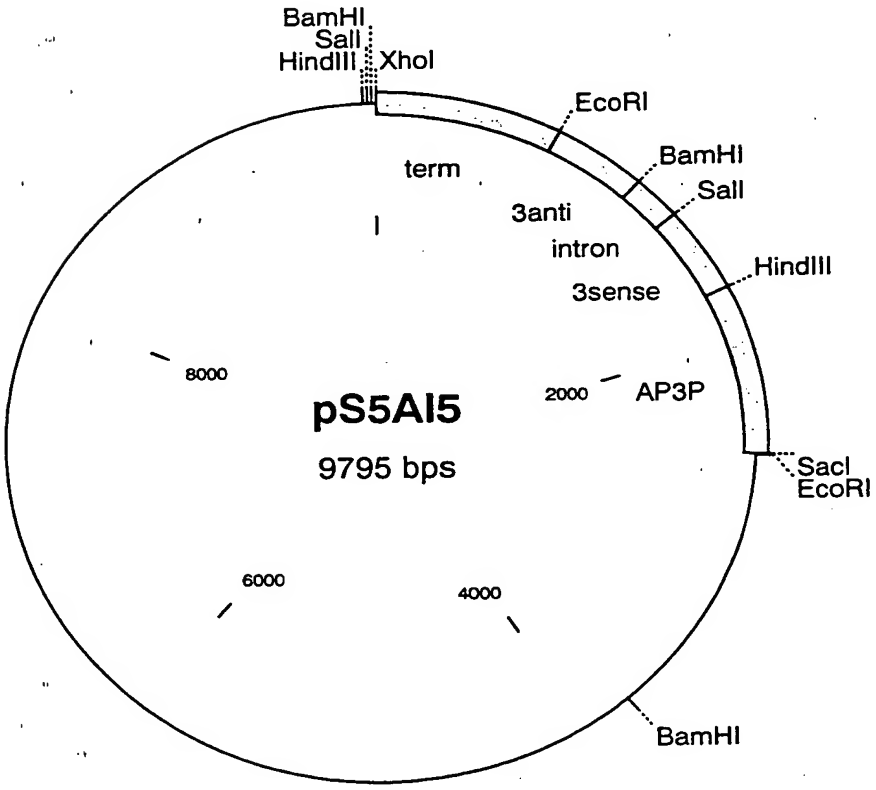


Figure 16: Inverse PCR amplificate comprising the 312 bp fragment of the epsilon-cyclase promoter

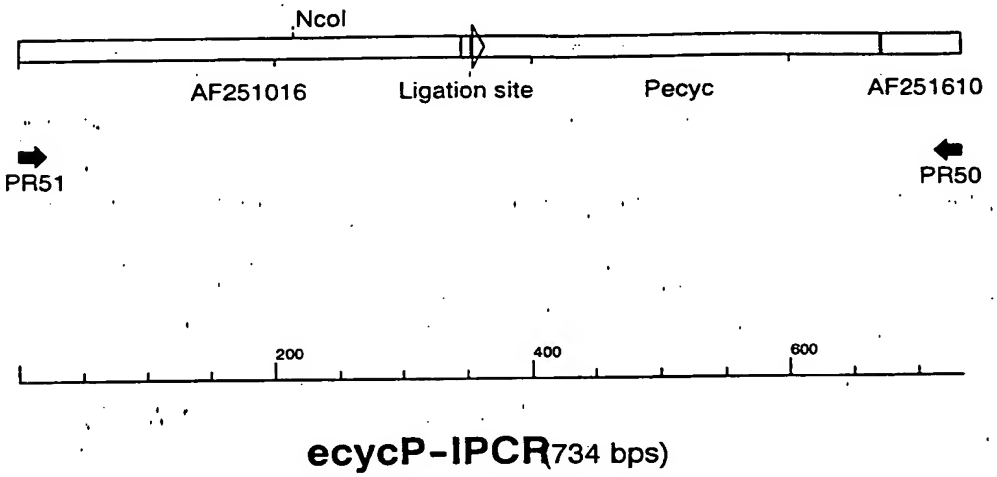


Figure 17: TAIL PCR amplificate comprising the 199 bp fragment of the epsilon-cyclase promoter

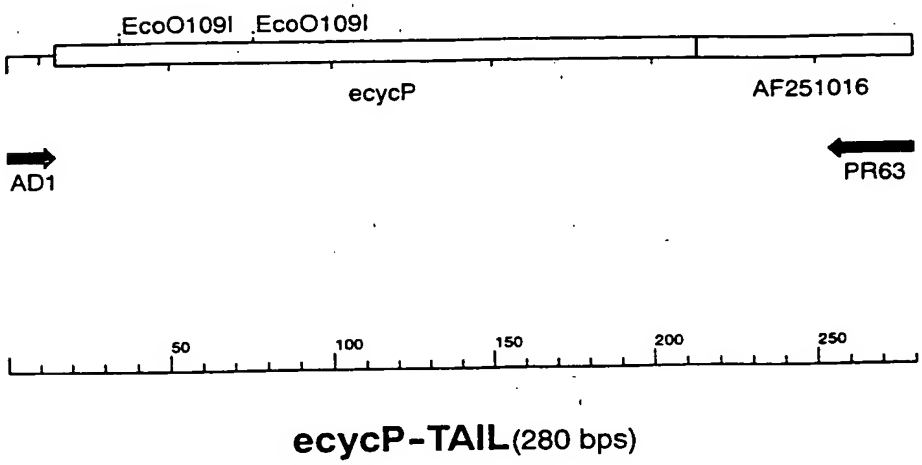


Figure 18: Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the AP3P promoter

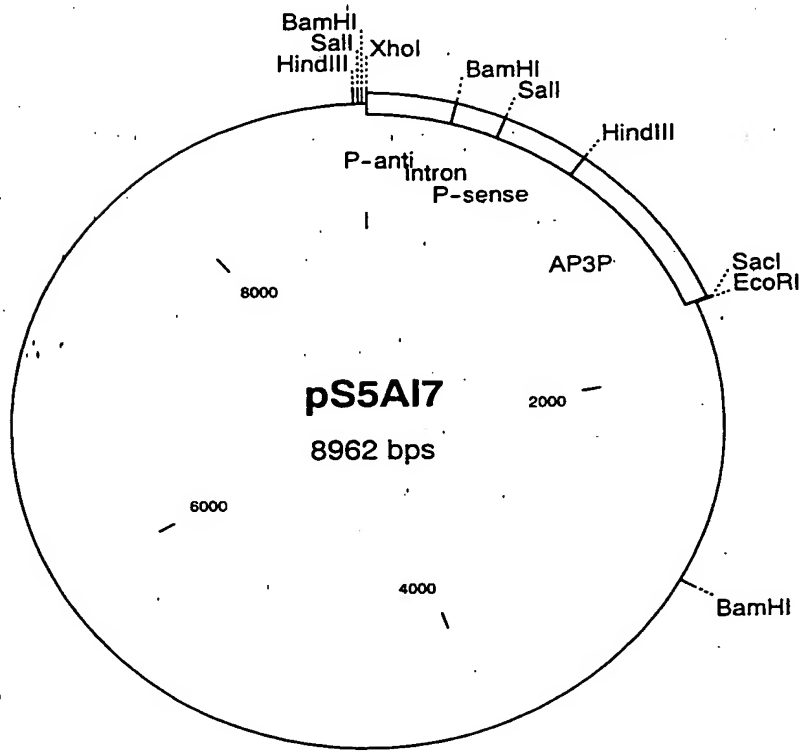


Figure 19: Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the CHRC promoter

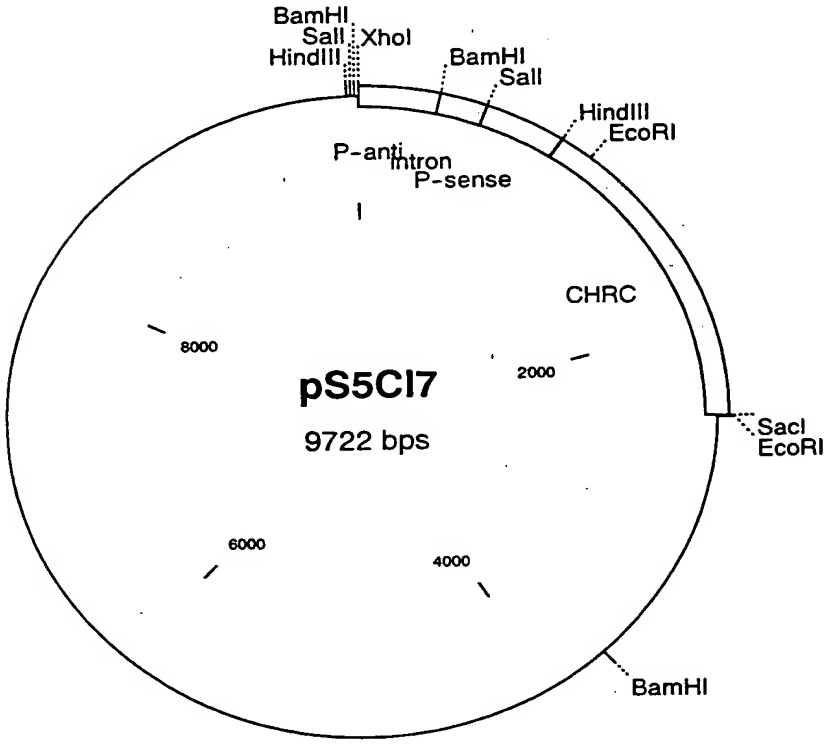


Figure 20: Expression vector for the flower-specific production of dsRNA transcripts comprising the 312 bp promoter fragment of the epsilon-cyclase under the control of the AP3P promoter and of the CHRC promoter

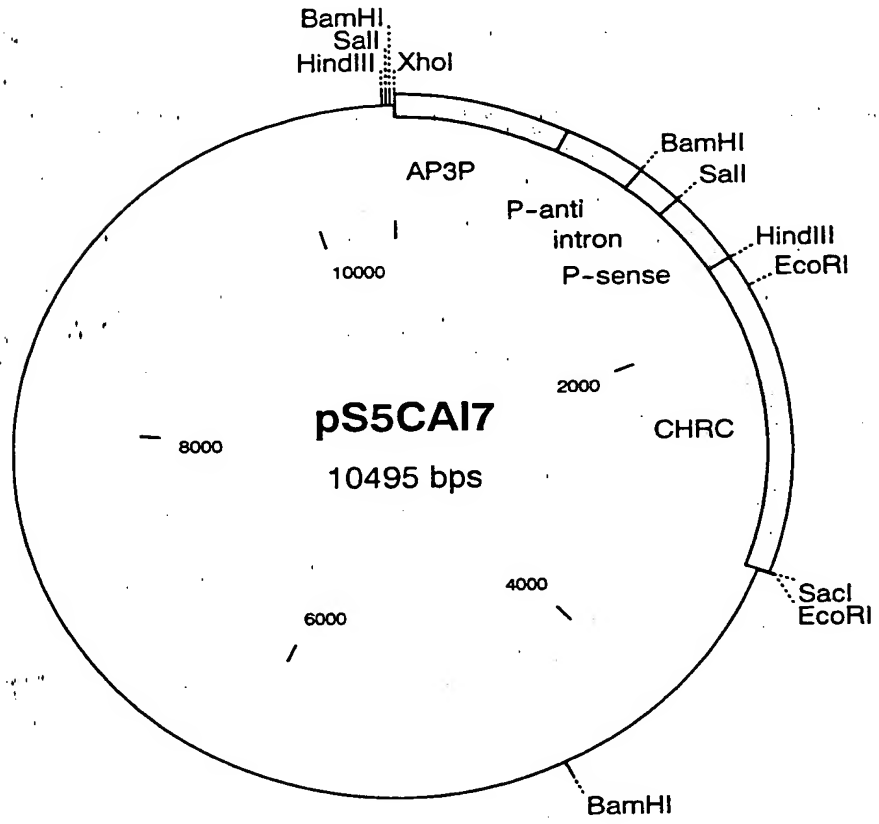
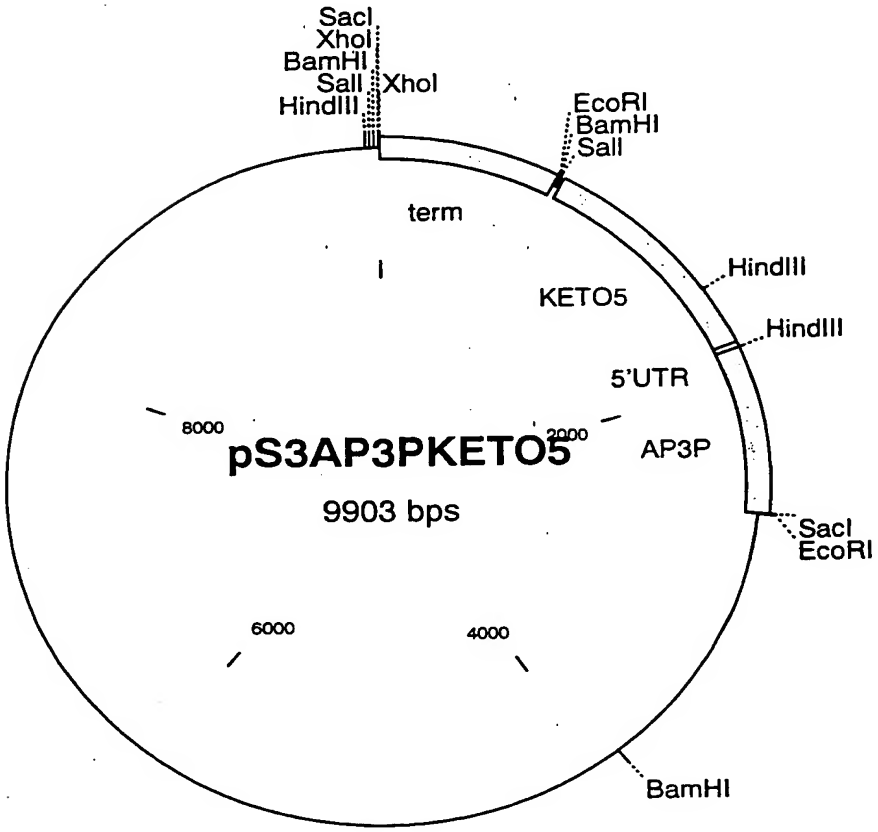


Figure 21: Construct for the flower-specific overexpression of the ketolase (β-C-4-oxygenase) protein from *H. pluvialis* without heterologous transit peptide.



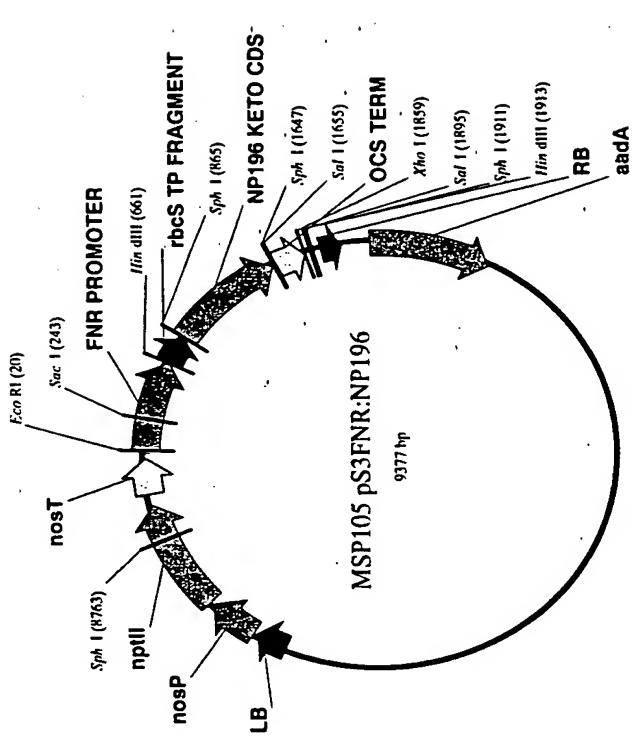


Figure 22: pSUN3 construct for overexpressing the β-C-4-oxygenase protein NP196 from *Nostoc punctiforme ATCC 29133* with rbcS transit peptide from pea under the control of the FNR promoter

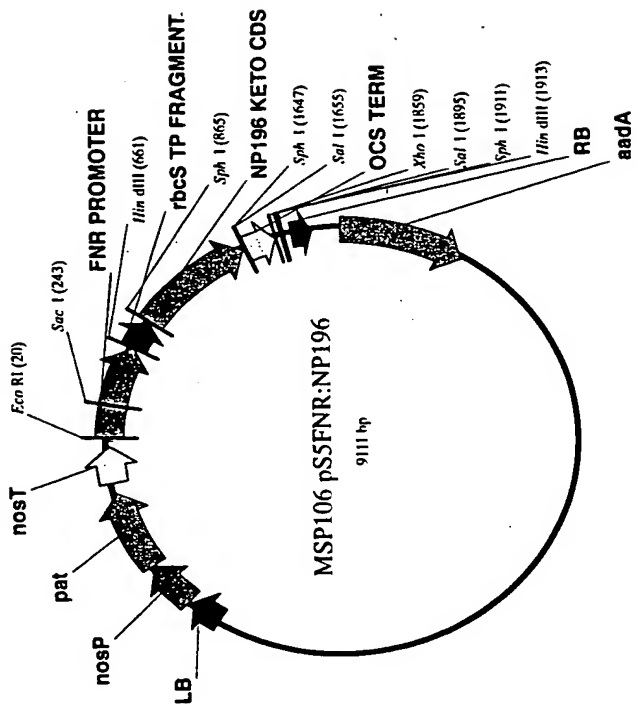


Figure 23: pSUN5 construct for overexpressing the β-C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter

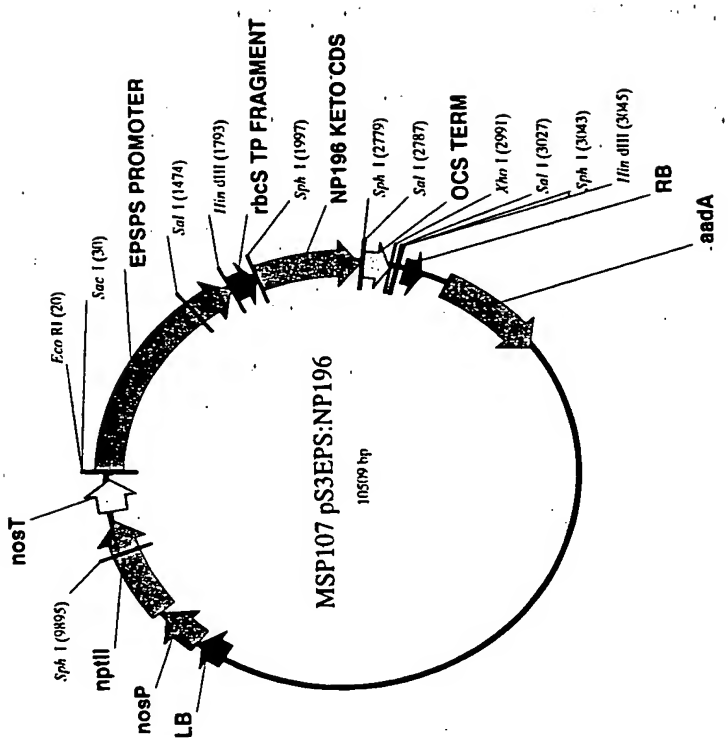


Figure 24: pSUN3 construct for overexpressing the β-C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter

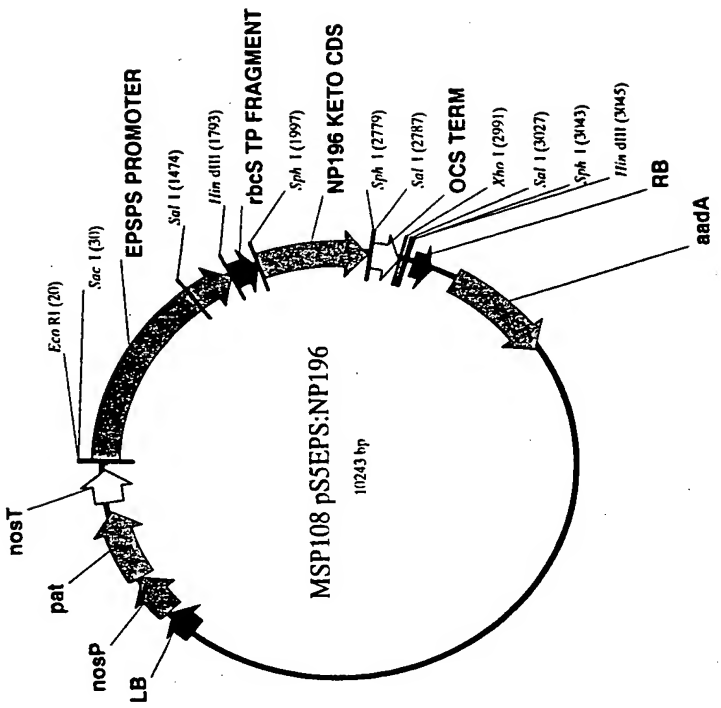


Figure 25: pSUN5 construct for overexpressing the β-C-4-oxygenase protein NP196 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter

Figure 26: pSUN3 construct for overexpressing the β-C-4-oxygenase protein NP195 from *Nostoc punctiforme ATCC 29133* with rbcS transit peptide from pea under the control of the FNR promoter

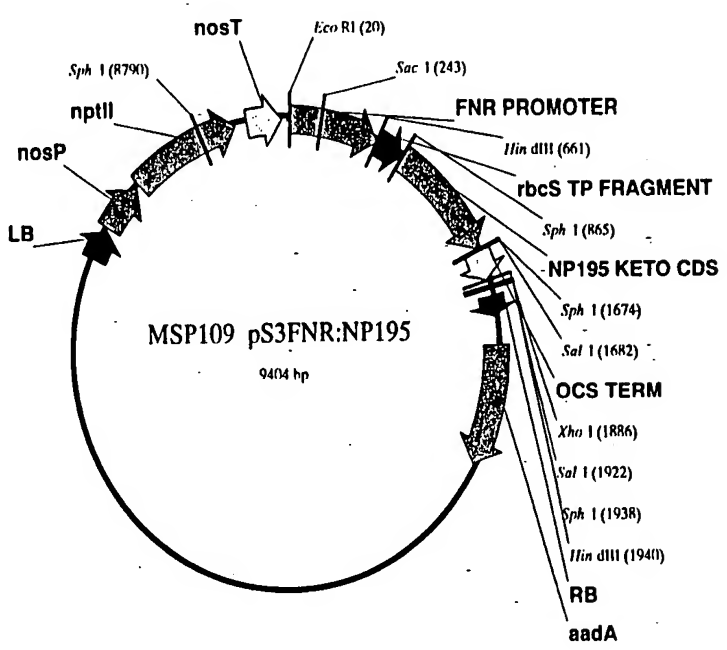
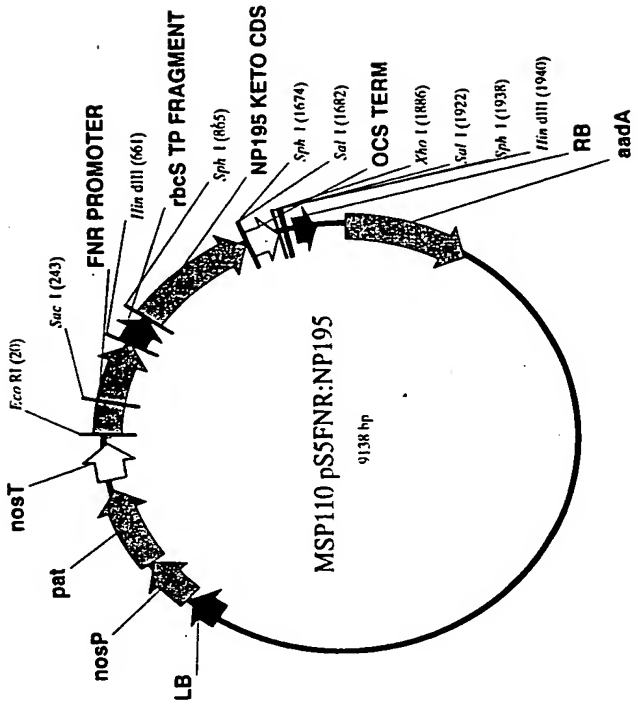


Figure 27: pSUN5 construct for overexpressing the β-C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the FNR promoter



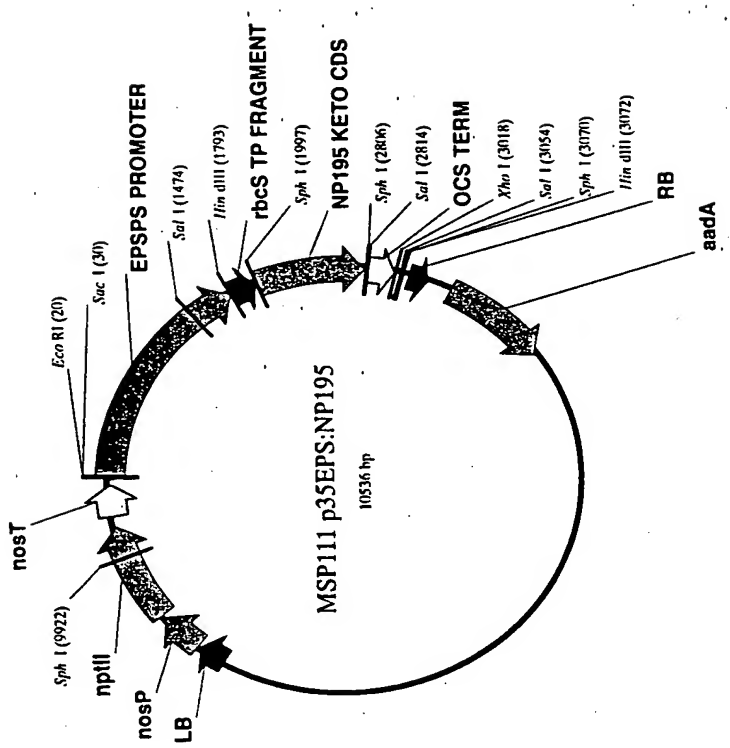


Figure 28: pSUN3 construct for overexpressing the β-C-4-oxygenase protein NP195 from *Nostoc punctiforme* ATCC 29133 with rbcS transit peptide from pea under the control of the EPSPS promoter

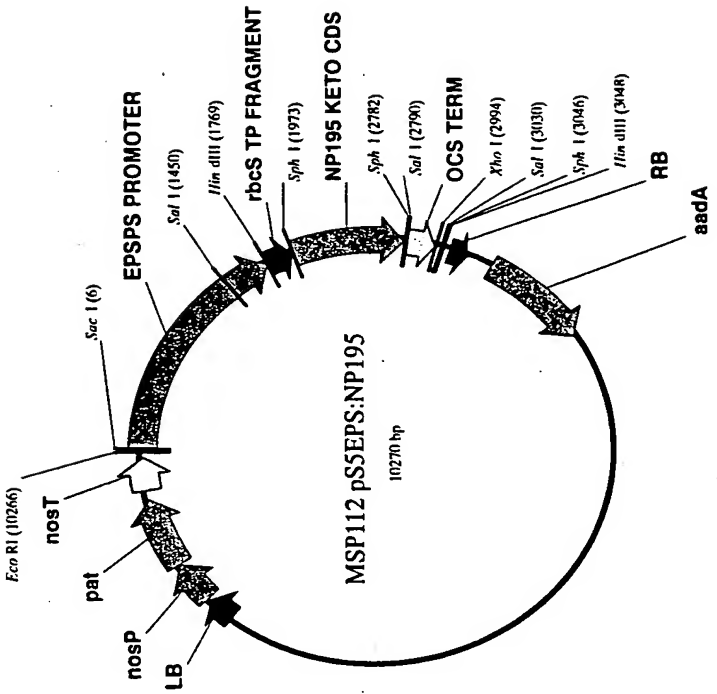


Figure 29: pSUN5 construct for overexpressing the β-C-4-oxygenase protein NP195 from *Nostoc punctiforme ATCC 29133* with rbcS transit peptide from pea under the control of the EPSPS promoter

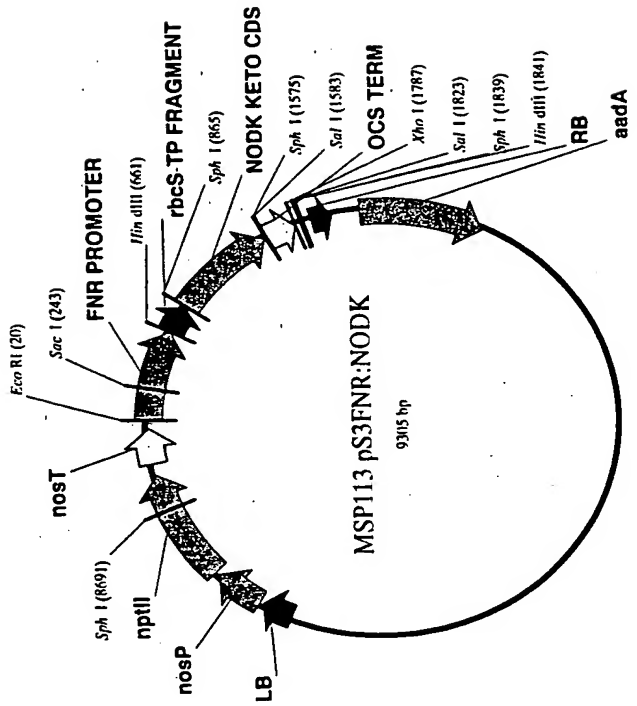


Figure 30: pSUN3 construct for overexpressing the β-C-4-oxygenase protein from *Nodularia spumigena* *NSOR10* with rbcS transit peptide from pea under the control of the FNR promoter

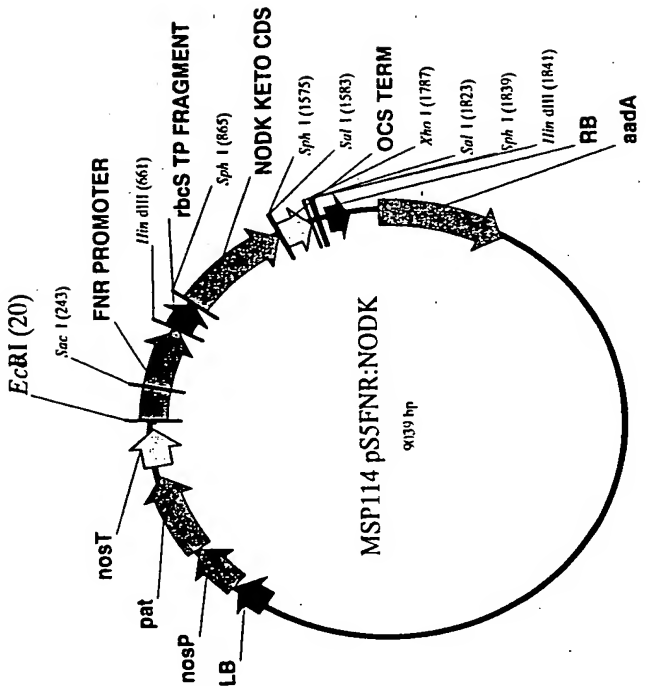
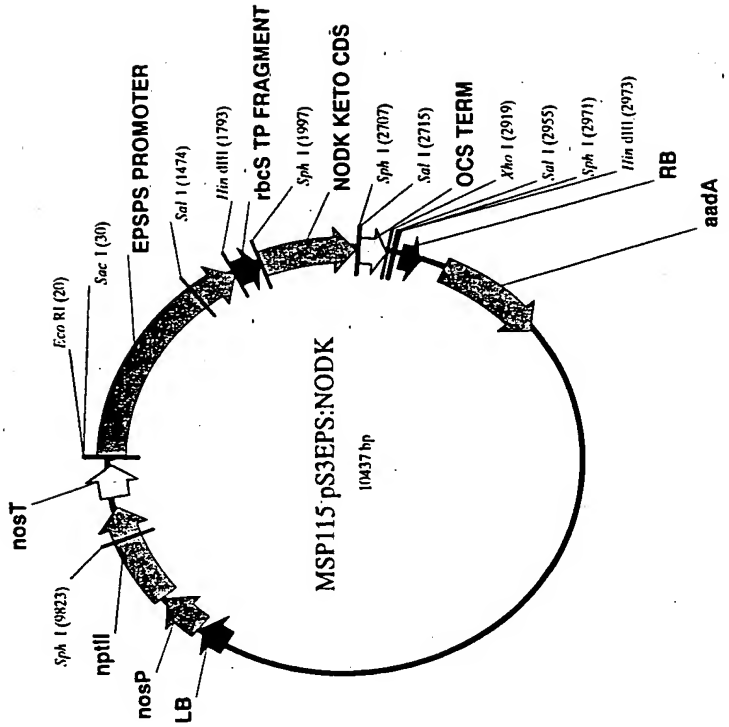


Figure 31: pSUN5 construct for overexpressing the β-C-4-oxygenase protein from *Nodularia spumigena NSOR10* with rbcS transit peptide from pea under the control of the FNR promoter

Figure 32: pSUN3 construct for overexpressing the β-C-4-oxygenase protein from *Nodularia spumigena NSOR10* with rbcS transit peptide from pea under the control of the EPSPS promoter



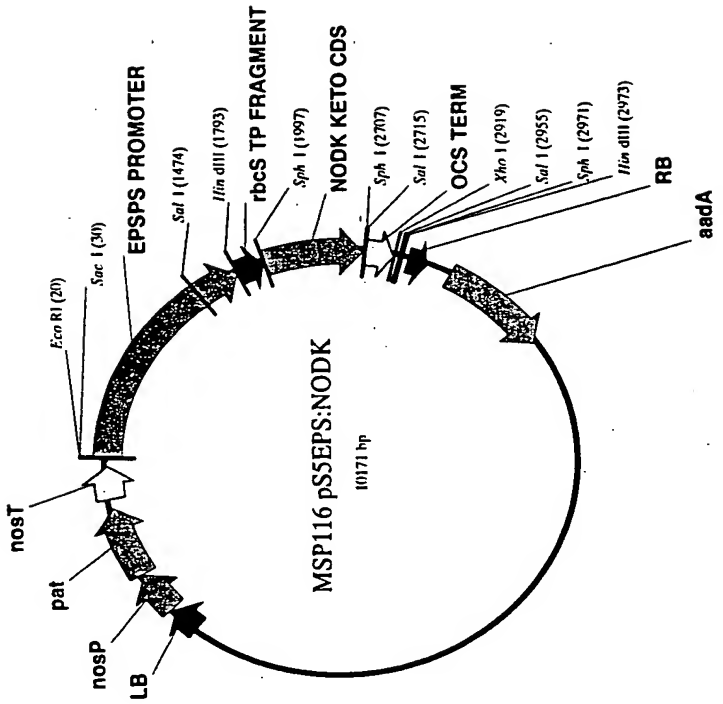


Figure 33: pSUN5 construct for overexpressing the β-C-4-oxygenase protein from *Nodularia spumigena NSOR10* with rbcS transit peptide from pea under the control of the EPSPS promoter

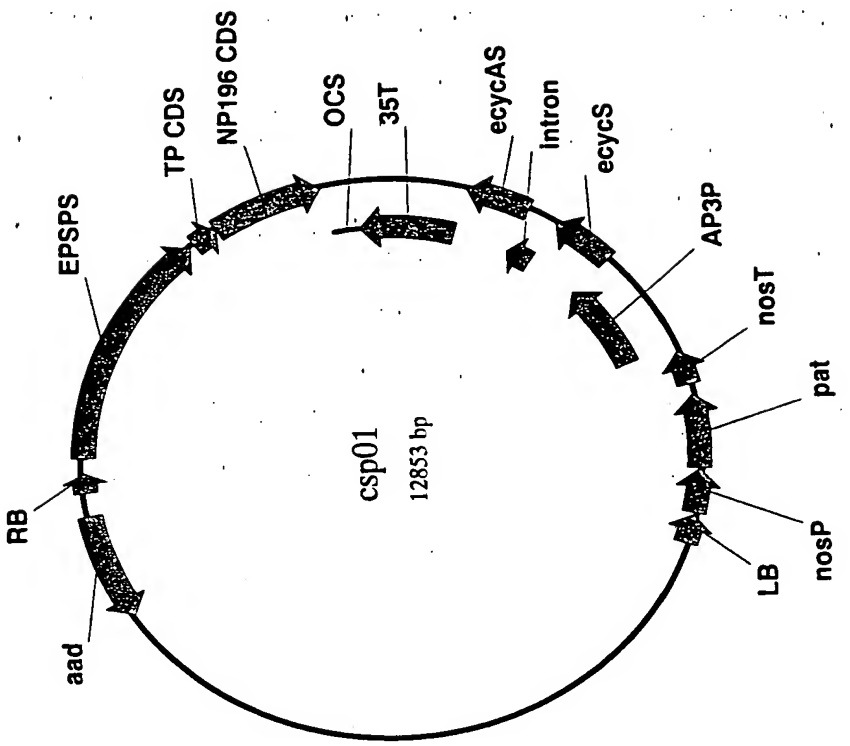


Figure 34: pSUN5 construct for overexpressing the β-C-4-oxygenase protein from *Nodularia spumigena NSOR10* and downregulating the endogenous Tagetes epsilon-cyclase in Tagetes erecta

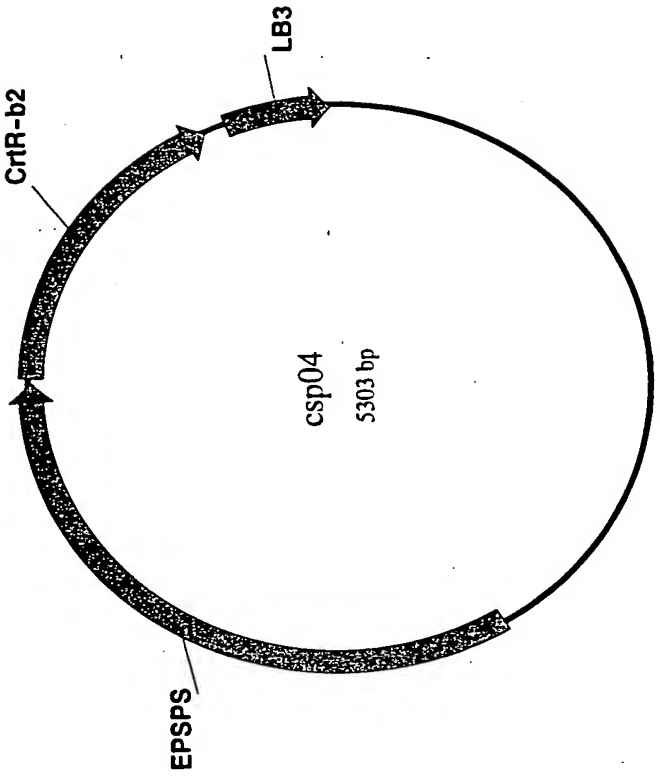


Figure 35: Expression cassette for overexpressing the β -hydroxylase from tomato under the control of the EPSPS promoter

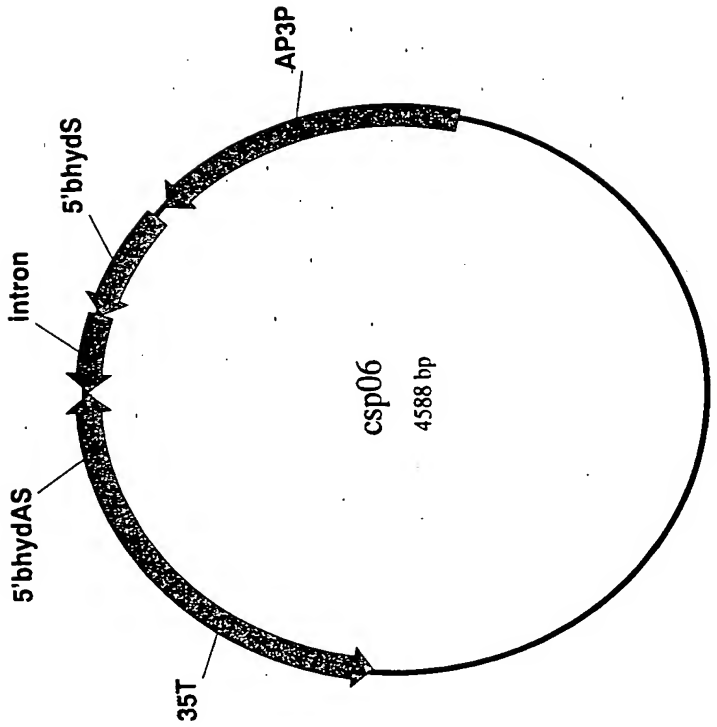


Figure 36: Expression cassette for downregulating the endogenous β -hydroxylase from Tagetes under the control of the EPSPS promoter

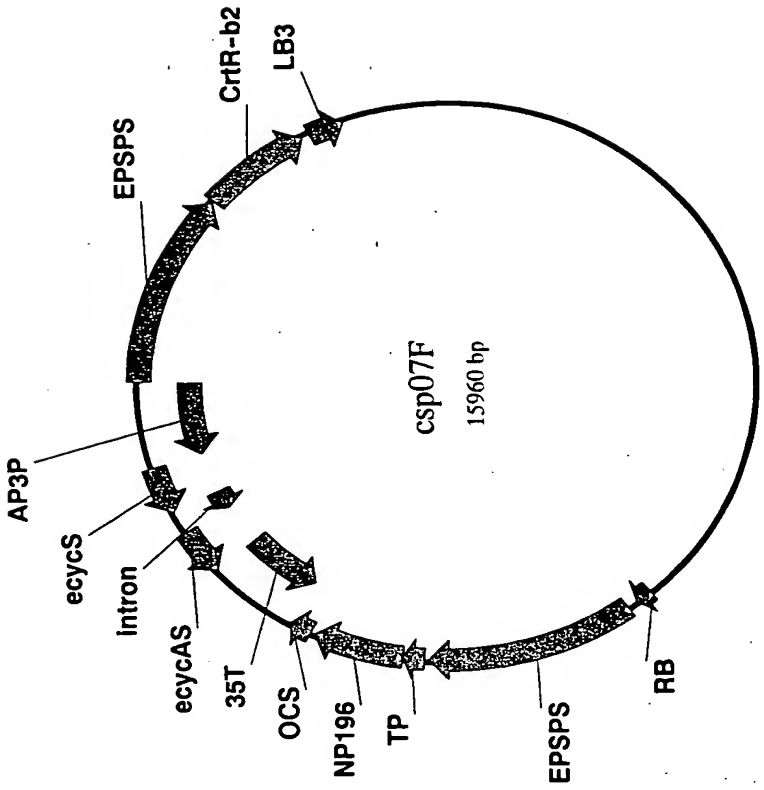


Figure 37: pSUN5 construct for downregulating the endogenous Tagetes epsilon-cyclase and overexpressing the NP196 ketolase and the tomato β-hydrolase

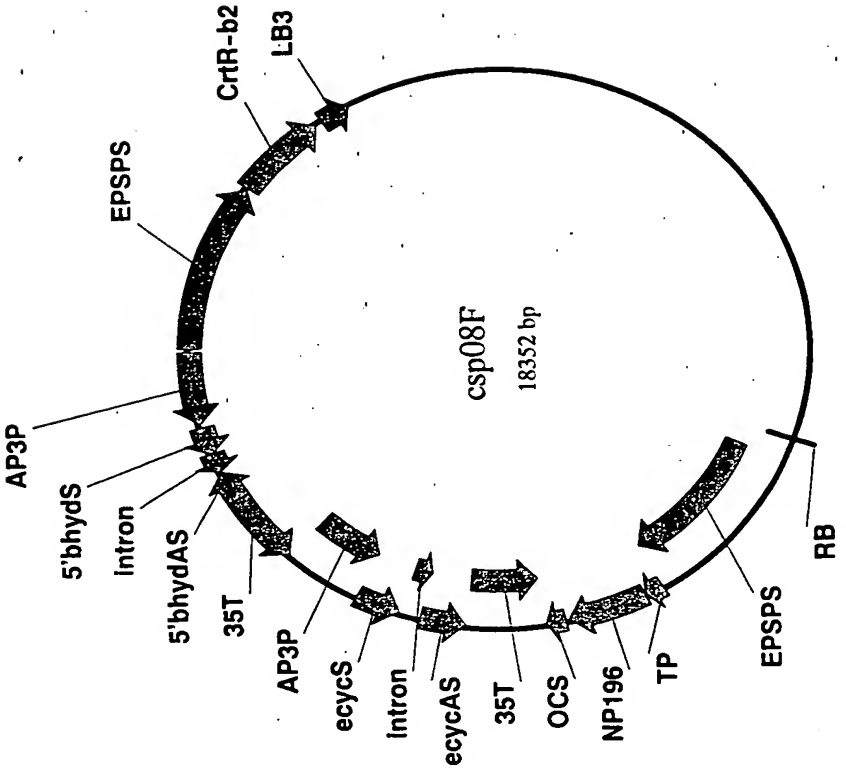


Figure 38: pSUN5 construct for downregulating the endogenous Tagetes epsilon-cyclase, overexpressing the endogenous Tagetes β -hydroxylase and overexpressing the NP196 ketolase and the tomato β-hydroxylase

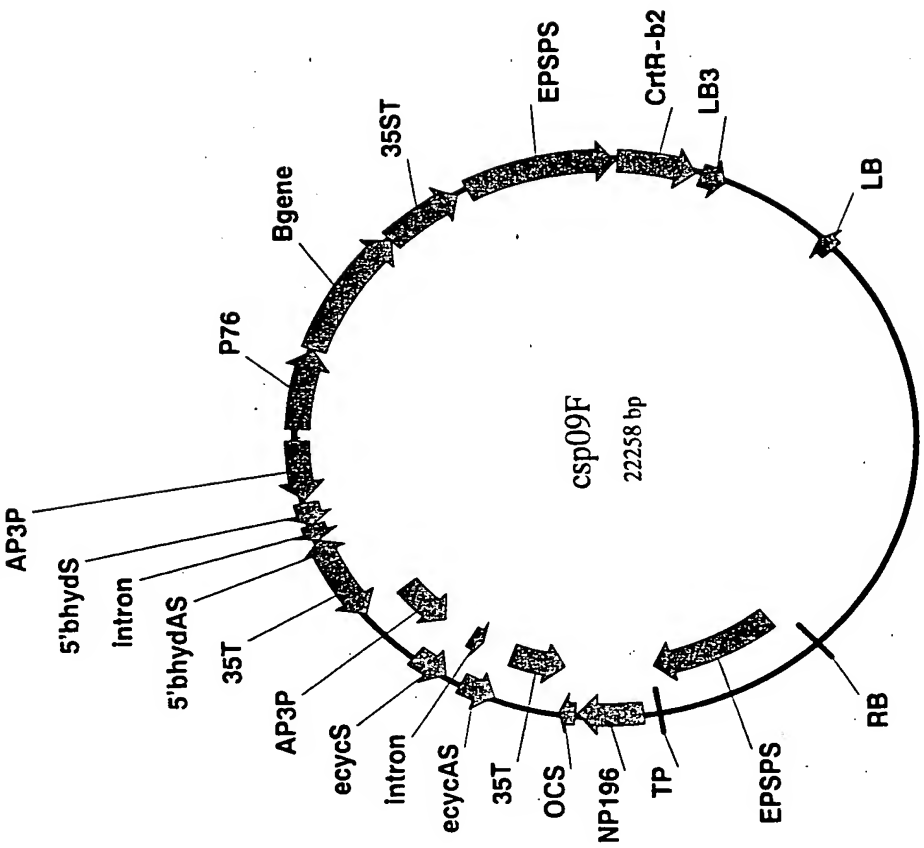


Figure 39: pSUN5 construct for downregulating the endogenous Tagetes epsilon-cyclase, downregulating the endogenous Tagetes β-hydroxylase and overexpresing the NP196 ketolase and the tomato β-hydroxylase and the B gene from tomato

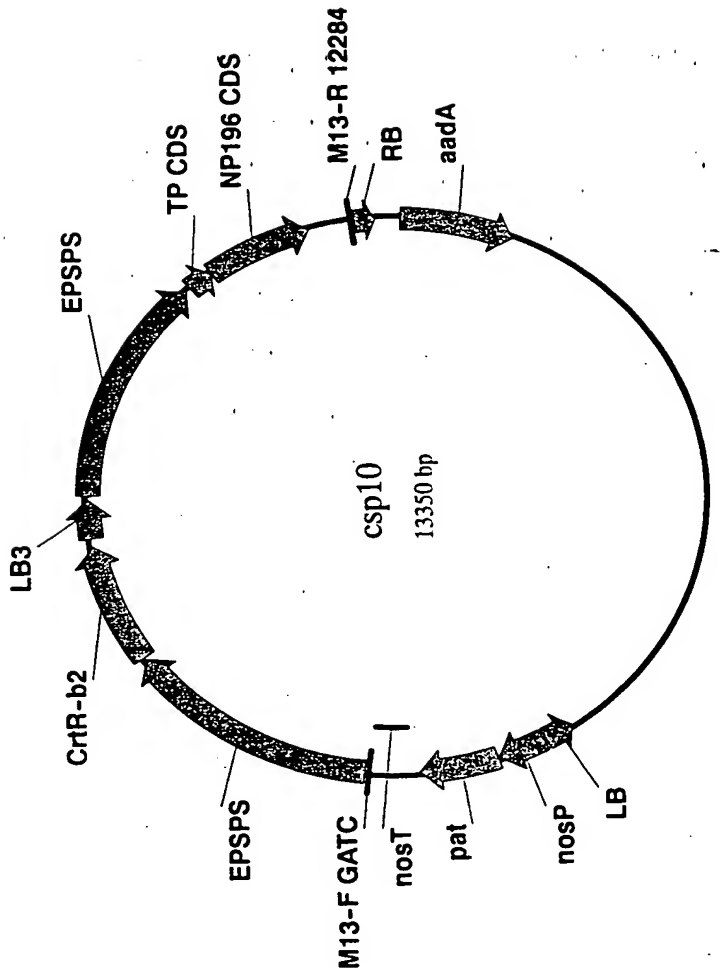


Figure 40: pSUN5 construct for overexpressing the NP196 ketolase and the tomato β-hydroxylase

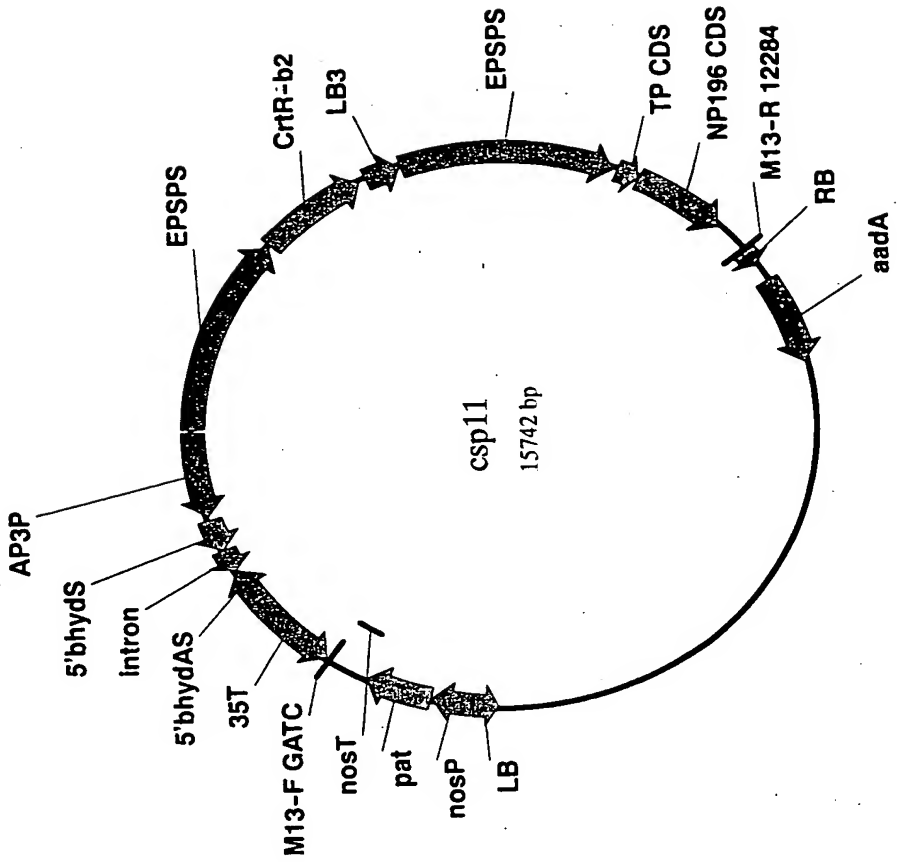


Figure 41: pSUN5 construct for downregulating the endogenous Tagetes β-hydroxylase and downregulating the NP196 ketolase and the tomato β-hydroxylase

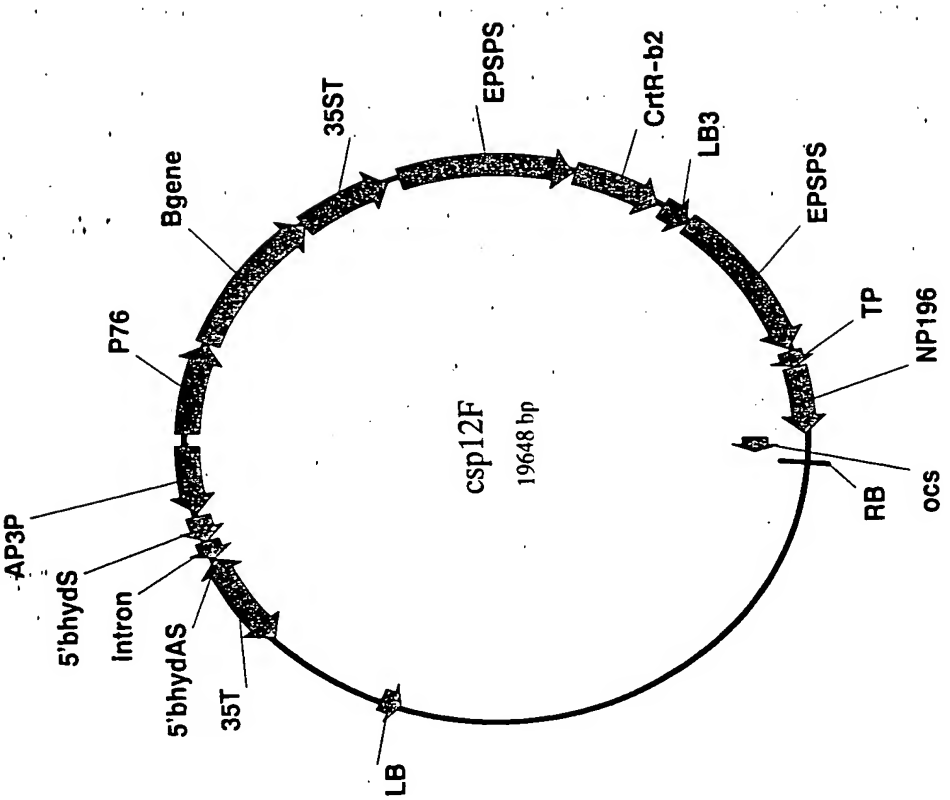


Figure 42: pSUN5 construct for downregulating the endogenous Tagetes β-hydroxylase and for overexpressing the NP196 ketolase, the B gene and the tomato β-hydroxylase

Figure 43: Expression vector for the flower-specific expression of the chromoplast-specific lycopene beta-cyclase from Lycopersicon esculentum under the control of the promoter P76 and for the flower-specific expression of the ketolase NP196 from Nostoc punctiforme ATCC 29133 under the control of the EPSPS promoter

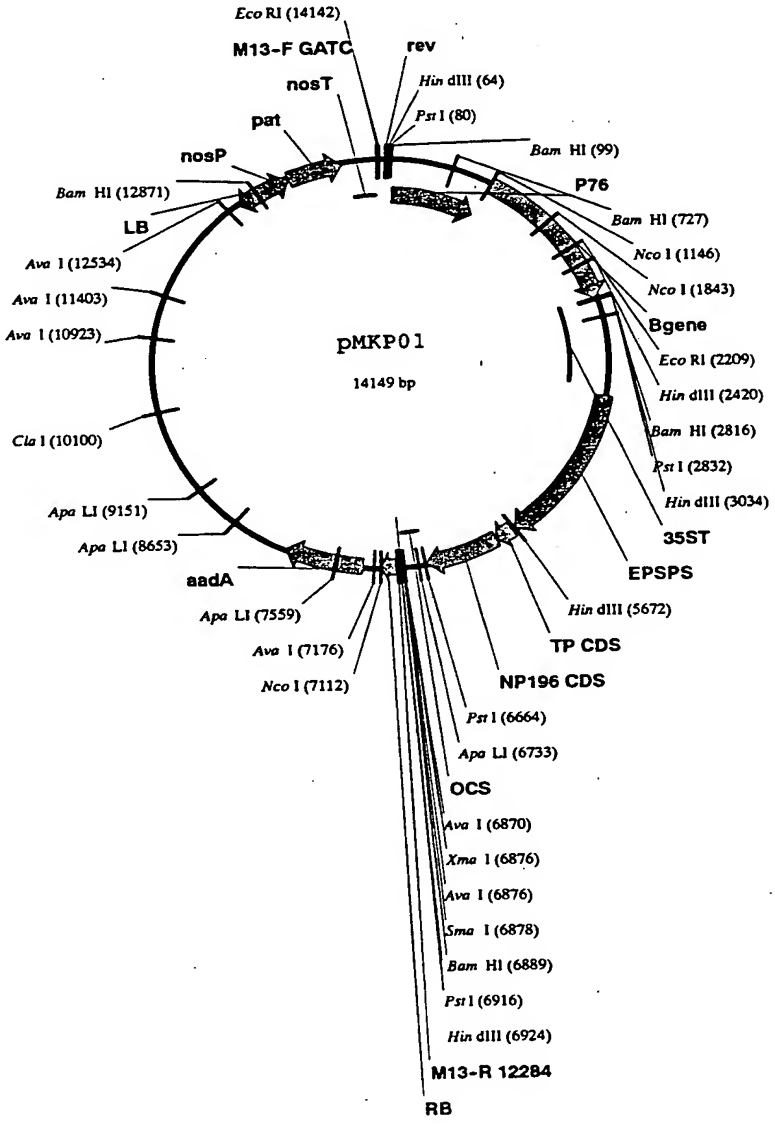


Figure 44: Expression vector for the flower-specific expression of the chromoplast-specific lycopene beta-cyclase from Lycopersicon esculentum under the control of the promoter P76, for the flower-specific expression of the ketolase NP196 from Nostoc punctiforme ATCC 29133 under the control of the EPSPS promoter and for the flower-specific production of dsRNA transcripts comprising 5'-terminal fragments of the epsilon-cyclase cDNA (AF251016) under the control of the AP3P promoter